# EFFECT OF HEPARIN ON CHROMATIN

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## 1. Introduction

Polyanions, including heparin, when added to nuclei, cause pronounced morphological changes [1–4] which have been correlated with increased DNA and RNA syntheses [5–8]. Heparin stimulates also RNA synthesis when added to isolated chromatin [7,8]. It was proposed that this stimulation results from a direct interaction of the anions with histones [2, 9–11].

In this work we report the effect of heparin on the individual proteins of chromatin. At low concentration heparin binds essentially to histones H2A and H2B when added to proteins extracted from chromatin, whereas it binds to all histones when added to chromatin. At a higher concentration heparin binds to all histones whether extracted or bound in chromatin. The binding of heparin to all non-histone proteins (NHP) requires a much higher heparin concentration, suggesting that at least part of the NHP is directly associated with DNA.

## 2. Materials and methods

### 2.1. *Preparation of chromatin and of chromatin proteins*

Liver chromatin was prepared from Wistar rats by the method of Reeder and Roeder [12] modified by De Pomerai et al. [13]. Proteins were extracted from solubilized chromatin by addition of 10 volumes of the extracting solution : 7 M urea, 3 M NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM β-mercaptoethanol [14]. DNA was then removed by a 48 h centrifugation in a SW 39 Beckman rotor at 35 000 rev/min.

### 2.2. *Extraction of chromatin protein by heparin*

Chromatin samples containing 10 mg of proteins were mixed with various amounts of heparin (Sigma) for 18 h at 4°C. After a 48 h centrifugation in a SW 39 rotor at 35 000 rev/min, the supernatant was taken out and an aliquot was submitted to electrophoresis. The pellet was then extracted with the extracting solution as described above. Proteins extracted from identical amounts of chromatin were analyzed by electrophoresis.

### 2.3. *Polyacrylamide gel electrophoreses*

Two types of electrophoreses were performed : (a) in the presence of 2.5 M urea according to the Panyim and Chalkley method [15] modified by Shaw and Huang [16]; (b) in the presence of 0.1% sodium dodecyl sulfate (SDS) according to Laemmli [17].

## 3. Results

### 3.1. *Effect of heparin on isolated chromatin proteins*

We have studied the interaction of heparin with isolated chromatin proteins by its effect in their electrophoretic mobility. Heparin was added to chromatin proteins at the ratios of 1:10, 1:4, 1:1 (w/w). Samples containing 200 μg of proteins were loaded on polyacrylamide gel and electrophoreses were performed either in the presence of urea according to Panyim and Chalkley, which allows the separation of histones from NHP (fig.1a), or in the presence of SDS according ot Laemmli.

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Heparin when added to proteins at a ratio of 1:10 has no significant effect on protein migration (fig.1b). When the ratio was 1:4, the histones H2A, H2B, part of H1 and H3 and a few classes of NHP did not migrate (fig.1c), which probably means that they were bound to heparin. With a 1:1 ratio, no protein migrated in the gel (fig.1d). In the electrophoreses performed in the SDS system, the migration of the proteins was identical in the presence and in the absence of heparin. It is then likely that heparin binds to extracted chromatin proteins and preferentially to H2A and H2B.

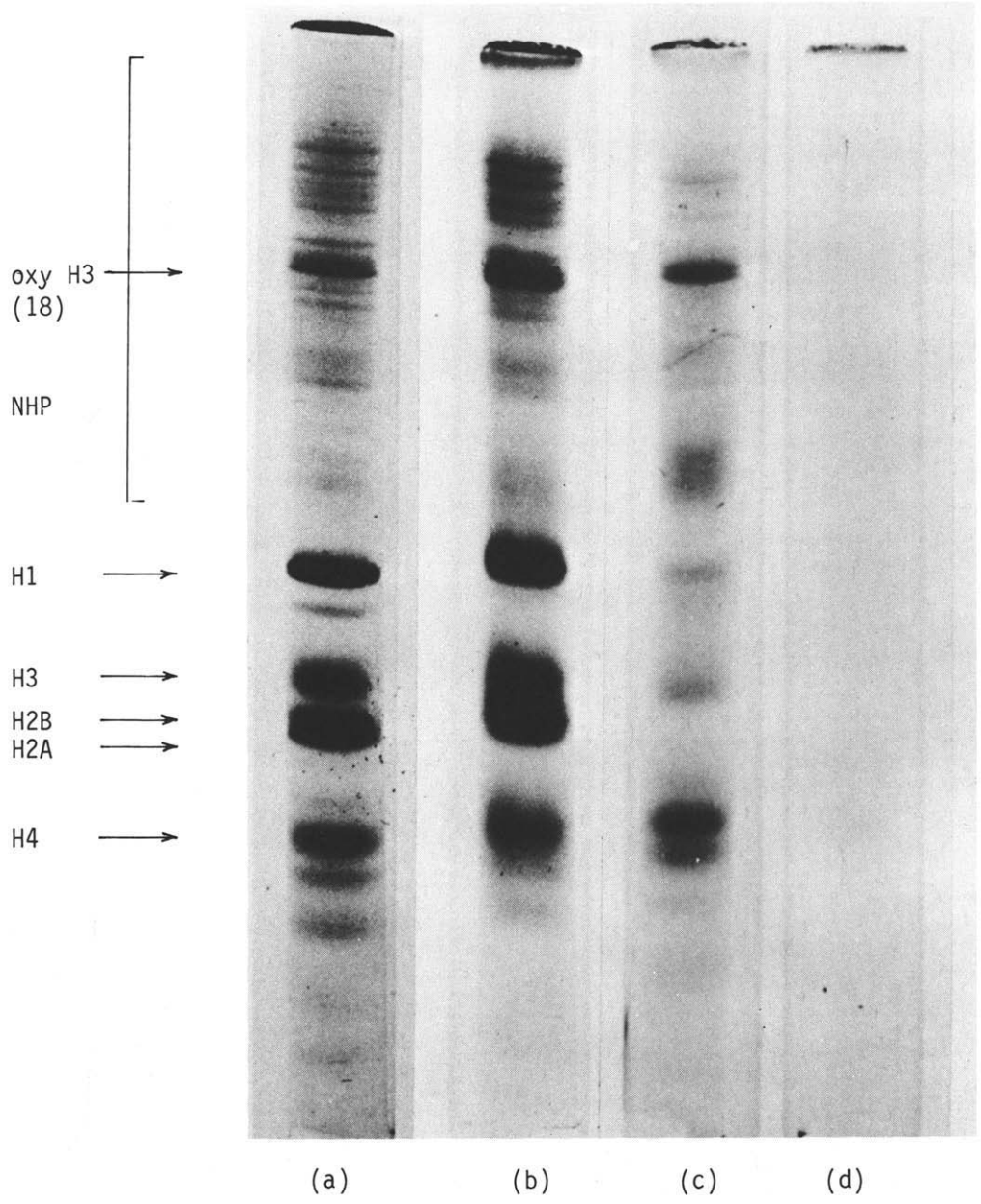


Fig.1. Effect of heparin on chromatin proteins. Electrophoresis according to Panyim and Chalkley. (a) Control in the absence of heparin. (b) Heparin–protein ratio 1:10. (c) Heparin–protein ratio 1:4. (d) Heparin–protein ratio 1:1.

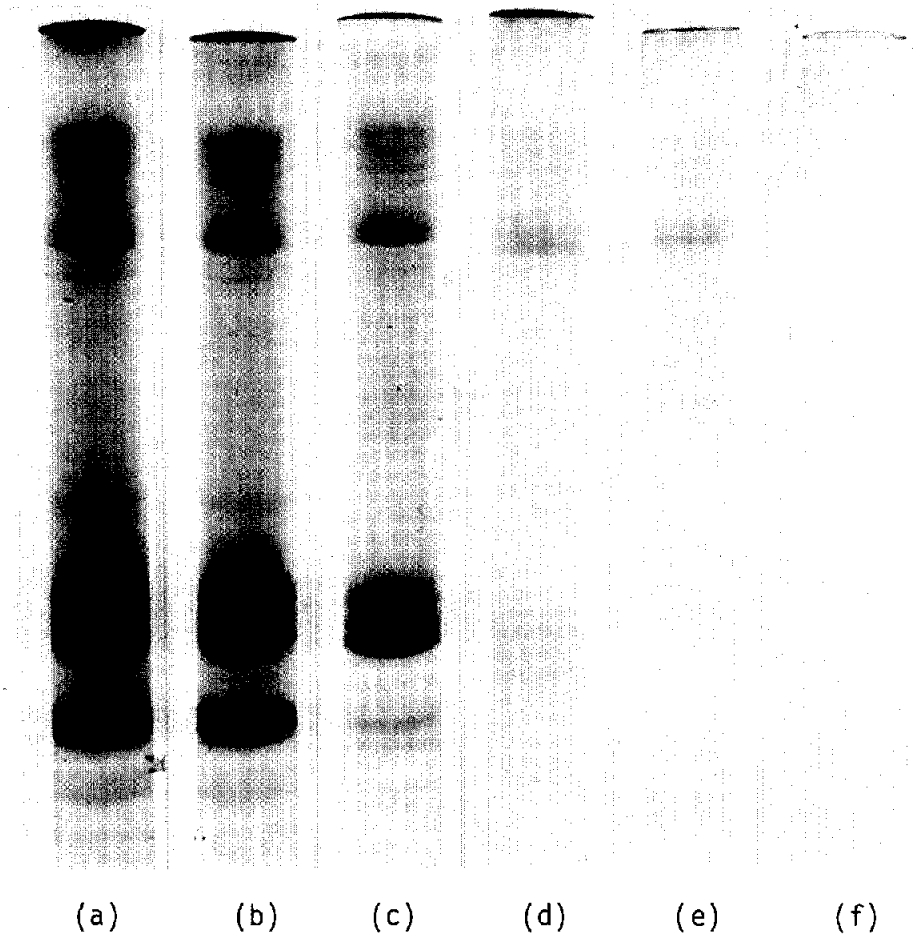


Fig. 2. Electrophoreses of the chromatin proteins left in chromatin after the extraction by heparin. Chromatin was first treated with various heparin concentrations and the proteins remaining in the chromatin were extracted by 7 M urea, 3 M NaCl and analyzed by electrophoresis performed according to Panyim and Chalkley. Proteins from non-treated chromatin (a). Proteins remaining after heparin treatment at heparin-protein ratios of: (b) 1:40; (c) 1:20; (d) 1:10; (e) 1:4; (f) 1:1.

### 3.2. *Effect of heparin on chromatin*

Liver chromatin was treated with increasing amounts of heparin with ratios of 1:40 to 1:1 (w/w). After centrifugation the proteins extracted by heparin were submitted to electrophoresis. In the urea system, no migration was observed whatever the heparin concentration used, indicating that heparin was bound to the extracted proteins.

The proteins remaining in the chromatin pellet were extracted with 7 M urea, 3 M NaCl and analyzed by electrophoreses performed according to Panyim and Chalkley.

Heparin at a 1:40 ratio did not extract proteins (fig.2b), since the electrophoretic pattern is identical to the pattern observed with chromatin non extracted with heparin (fig.2a). Heparin at a 1:20 ratio extracted histone H1, most of H4 and part of the other histones (fig.2c). Heparin at a 1:10 ratio extracted most of the histones and part of the NHP (fig.2d). Heparin at a 1:4 ratio also removed all histones (fig.2e). All proteins were extracted by heparin at a 1:1 ratio (fig.2f).

The electrophoretic pattern in the SDS system of the proteins extracted with heparin at a 1:1 ratio is identical to the pattern observed with the urea-NaCl

extract from chromatin not previously treated with heparin.

## 4. Discussion

In this work we have shown that heparin added at low concentration to isolated chromatin proteins binds preferentially to histones H2A and H2B. When added at the same concentration to chromatin, heparin removes the four histones H2A, H2B, H3 and H4, suggesting that the conformation of chromatin favors the cross-binding of heparin to several histone molecules. Other investigators using polystyren sulfonate [11] and SDS [19] have selectively extracted different classes of histones.

A 10-fold increase in heparin concentration is required for the extraction of most of the NHP from chromatin. It is then likely that many NHPs are directly bound to DNA, although we cannot exclude that the removal of histones produces some rearrangement of proteins on DNA. A direct binding of most of the NHP to DNA was also suggested by Courtois et al. [20].

The effect of various heparin concentrations on the thermal stability of chromatin is currently under investigation.

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